

Supporting Information

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SI Materials and Methods

The “Massimo Sella” Archive. The “Massimo Sella” archive was collected by one of the authors (M.S.) at the Istituto Italo-Germanico di Biologia Marina/Deutsch-Italienisches Institut für Meeresbiologie of Rovigno, Italy (now Institute Centre for Marine Research, Rovinj, Croatia). The Massimo Sella archive now at the Laboratory of Marine Biology and Fisheries, University of Bologna, Fano, Italy, includes more than 6,000 individual skeletal specimens (dried caudal vertebrae and fins) of juvenile and adult fish of Mediterranean large pelagic species (e.g., *T. thynnus*, *Thunnus alalunga*, *Euthynnus alletteratus*, *Sarda sarda*, *Xiphias gladius*) caught in Italian, Spanish, and North African tuna traps of the CWM (Fig. S1 and Table S1).

Sampling and Biological Data. Sampling and biological data of historical and contemporary samples of ABFT from the CWM are summarized in Table S1. Age class of all ABFT individuals was assessed based on the findings of Cort (1), except for that of the HADR and HCWM samples. The age class of HADR individuals was assessed by vertebral ring counting in a subset of 10 individuals. Age marks were read on caudal peduncle vertebrae, which were sawed along a sagittal plane. Sections were stained using silver nitrate staining (2). The age class of the HCWM individuals was estimated by calculating the fork length (FL), using the equation $HCF = 0.299FL - 2.123$ given by Cort (3), where HCF is the height of caudal fin. Individual HCF was measured from historical caudal fin specimens (Fig. S1B). Most individuals were adults and juveniles, whereas only one sample was formed by young-of-the-years (CLIG, year sample 2000). Three samples (HADR, CADR, and CLIG) were obtained from two to three annual sampling replicas. Annual replicas were pooled for the genetic data analyses after testing the lack of significant interannual genetic differentiation (estimated as pairwise F_{ST} : $F_{ST}^{HADR\ 1926-1927} = 0.013$, not significant; $F_{ST}^{CADR\ 2003-2004} = 0.007$, not significant; $F_{ST}^{CADR\ 2003-2005} = 0.023$, not significant after Bonferroni sequential correction; $F_{ST}^{CADR\ 2003-2005} = 0.022$, not significant after Bonferroni sequential correction; $F_{ST}^{CLIG\ 1999-2000} = -0.003$, not significant).

Historical DNA Extraction Protocol. To validate ancient DNA data, all extractions were independently replicated. DNA extraction and PCR amplification were carried out in a dedicated laboratory in which no modern DNA had ever been manipulated. For DNA extraction from the vertebrae, we used an ad hoc modified extraction protocol, and each extraction was replicated twice as recommended by Caramelli et al. (4). Because of heavy contaminations from bacteria and fungi, historical DNA analyses require thorough decontamination of samples and supplies. We cleaned surfaces and all supplies by wiping them with absolute ethanol and then by irradiating them with UV light (365-nm wavelengths). The surface of dried vertebrae was brushed and then irradiated with UV light, and, finally, the external part was cut with a hacksaw and discarded. Between different applications, all supplies were carefully washed with 0.4% NaOH solution and then rinsed with bidionized water. We cut the vertebral body into small cubes until a central core was obtained and then reduced this central part into fine bone powder. We decalcified the bone powder by incubating it overnight in a shaker at room temperature in 1.6 mL of EDTA buffer (0.5 M, pH 8.0). The next day, samples were precipitated by centrifugation at $550 \times g$ for 20 min and the decalcifying solution was discarded. After adding 1.6 mL of extraction buffer (0.1 M

EDTA, 0.5% N-laurylsarcosine-Na salt, 100 mg/mL Proteinase K) to the decalcified powder bone, samples were incubated overnight at 44°C in a shaker. After centrifugation at $12,500 \times g$ for 10 min at room temperature, 250 μ L of extraction solution was transferred in another microtube and 3.5 μ L of 1 μ g/ μ L Dextran Blue, 250 μ L of 4M NH₄-acetate, and 500 μ L of 96% vol/vol ethanol were added. To avoid the precipitation of inhibitors, we modified the precipitation procedure from the original protocol (5) by precipitating DNA on ice for 10 min and then centrifuged at $17,000 \times g$ for 15 min at 4°C. DNA was then washed with 250 μ L of 70% vol/vol ethanol and centrifuged at $17,000 \times g$ for 5 min at 4°C. DNA was resuspended in 30 μ L of deionized sterile water and stored at -20°C .

Contemporary DNA Extraction Protocol. Total genomic DNA from contemporary ABFTs was extracted from ethanol-stored specimens of soft tissues (fin, white skeletal muscle, and gill) according to a standard Cetyl Trimethyl Ammonium Bromide (CTAB) protocol (6).

Microsatellite Marker Analysis. Eight microsatellite loci [*T-tho4* (7); *Tth5*, *Tth10*, and *Tth34* (8); *Tth1-31*; *Tth208*; *Tth157*; and *Tth62* (9)] were selected as markers for genetic analysis according to two criteria. First, we chose loci with amplified alleles falling within a small size range so as to increase the quality of amplification of presumably degraded historical DNA. Second, we chose loci that are generally easy to amplify and, hence, produce results that can easily be replicated in both historical and contemporary samples. Particular care was taken in avoiding technical artifacts potentially leading to erroneous genotyping, including the effects of contamination, allele dropout, and detection of false alleles. To rule out contamination by exogenous DNA, we had negative controls for all DNA extraction and amplification steps. To ensure that the data could be reproduced, PCR and genotyping were replicated five times for each locus in 20% of the historical samples. The accuracy of allele scoring was tested by sequencing PCR products at all loci in a subset of 20 individuals. Microsatellite loci were amplified by using the following PCR conditions: 3-min denaturation at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at annealing temperature, and 30 s at 72°C for extension. An extra final extension of 3 min at 72°C was added after the last cycle. All ancient DNA PCR reactions were carried out in a 25- μ L reaction containing 10 mM Tris-HCl (pH 9), 50 mM KCl, 0.1% Triton X-100, 1.5 mM MgCl₂, 0.4 mM each dNTP, 1 μ M each primer, 160 μ g/mL BSA (BioLab), 2.5 U of Taq DNA Polymerase (Promega), and 50 ng of genomic DNA. Amplifications of modern DNA were carried out in a 10- μ L reaction of 10 mM Tris-HCl (pH 9), 50 mM KCl, 0.1% Triton X-100, 1.5 mM MgCl₂, 0.2 mM each dNTP, 0.5 μ M each primer, 0.5 U of Taq DNA Polymerase, and 20 ng of genomic DNA. To genotype individuals, we assessed the allele size on an ABIPrism 310 automatic sequencer (Applied Biosystems) using the labeled forward primers (6-FAM, HEX, or TAMRA; MWG Biotech) and the ROX 500 (Applied Biosystems) as an internal standard. Allele sizing was determined by analyzing PCR products using GeneScan Analysis v. 2.02 software (Applied Biosystems).

Bottleneck Analysis. According to the test proposed by Cornuet and Luikart (10), we compared the H_{cs} with their H_{ec} s estimated from the allele number, since during a bottleneck allele number is reduced faster than the heterozygosity. Simulations under the standard coalescent model [BOTTLENECK version 1.2.02 (11)] generated equilibrium distributions of H_{ec} reflecting the number

of alleles and the sample size under three mutation models, namely, IAM (12), SMM (13), and TPM (14). For TPM, we set multistep mutation events = 5% and variance = 12, as in the study by Piry et al. (11). For each mutation model, we finally compared expected heterozygosities and expected heterozygosities simulated from the allele number at equilibrium by the Wilcoxon test (one- and two-tailed). $H_e > H_{eq}$ means a heterozygosity excess and suggests a bottleneck; demographic growth has the opposite effect, and $H_e < H_{eq}$ (10).

In the test and software (M_P_Val) developed by Garza and Williamson (15), the average \bar{M} value was calculated across loci and compared with the critical value (M_{crit}) estimated through 10,000 simulations with the same parameters as the data (sample sizes, p_s , Δg , θ) but assuming the population to be at drift-migration equilibrium. For each simulation, the M ratio is calculated obtaining an empirical distribution; M_{crit} is defined as the fifth percentile of that distribution. We examined a range of

mutation models [as recommended by Garza and Williamson (15)] and chose conservative values for the unknown parameters p_s (frequency of multistep mutations) and Δg (mean size of non-one-step mutations): $p_s = 0.1$ and $\Delta g = 3.5$ (15). For each scenario, we used four tentative θ values, namely, (a) and (b), the previously estimated short-term and long-term values, and (c) and (d), two values often observed in natural populations ($\theta = 0.5$ and $\theta = 10$) (16).

Results

The historical sample, HCWM, showed an unusual genotype distribution, with a strong excess of heterozygotes at many loci (Table S1). For instance, we observed 85 copies of allele *2 at locus Tth157 and 60 copies of allele *2 at locus Tth10, but no *2*2 homozygotes were observed at either locus. In view of this, and of the impossibility of logically justifying these results, we decided to disregard this sample in all successive analyses.

1. Cort JL (1991) Age and growth of the bluefin tuna *Thunnus thynnus* (L.) of the Northeast Atlantic. *Collective Volume of Scientific Papers* 35:213–230.
2. Stevens JD (1975) Vertebral rings as a means of age determination in the blue shark (*Prionace glauca* L.). *J Mar Biol Assoc UK* 55:657–665.
3. Cort JL (1990) Biology and fishery of the Atlantic Bluefin tuna, *Thunnus thynnus*, in the Cantabric Sea (Translated from Spanish). *Publicaciones Especiales IEO 4 (Malaga, Spain)*.
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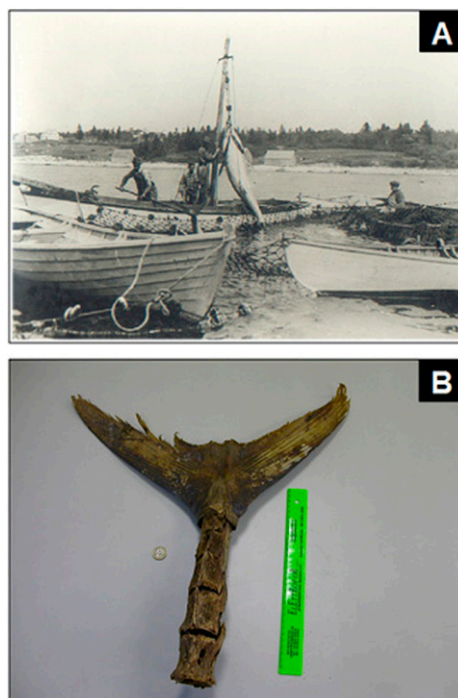


Fig. S1. Historical records of the ABFT fishery in the CWM from the personal archive of one of the authors (M.S). (A) Picture recorded at the beginning of the 20th century showing the “mattanza” of Giant Bluefin tunas in an STY trap. (B) Caudal fin of a historical Giant Bluefin tuna used for DNA analysis.

Table S1. Sampling and biological data of the historical and contemporary *T. thynnus* samples

Sample	Location	Area or geographical coordinates	Sampling year, period, date	Gear class	n	FL (cm)	Age classes*	Specimen type
HADR	Adriatic Sea [†]	North Adriatic Sea	1926	Tuna trap	57	NA	2 [‡]	Caudal vertebrae
		North Adriatic Sea	1927	Tuna trap	12	NA	2 [‡]	Caudal vertebrae
HSTY	Southern Tyrrhenian Sea	Messina shore (Italy)	1911, April 19–September 12	Tuna trap	39	88–112	2–4	Caudal vertebrae
HCWM	Central Western Mediterranean	unknown	1911–1926	Tuna trap	111	97–221 [§]	4–12	Caudal vertebrae
CADR	Adriatic Sea [†]	Central Adriatic Sea	2003, 14–28 April	Long-line	38	107–210	5–11	Finclip
		Central Adriatic Sea	2004, 25–26 August	Long-line	24	142–260	6–17	Finclip
		North Adriatic Sea	2005, 17–29 August	Long-line	11	122–170	5–8	Finclip
CSTY	Southern Tyrrhenian Sea	Southern Tyrrhenian Sea	2007, 20–29 August	Purse seine	39	107–287	4–24	Muscle
CLIG	Ligurian Sea [†]	Camogli shore (Italy)	1999, September 29–October 13	Purse seine	12	68–112	2–4	Finclip
		Camogli shore (Italy)	2000, April 26	Purse seine	24	39–52	1	Finclip
CSAR	Southwestern Sardinia	Carlo Forte Island (Italy)	2005, 21–26 May	Tuna trap	29	121–262	5–18	Muscle
CALG	Algerian coast	36° 51' N–01° 47' E 37° 32' N–06° 22' E	2006, April 21–May 20	Long-line	39	110–260	4–18	Finclip
CALB	Alboran Sea	Puerto Mazarron (Spain)	2005, 6–8 July	Long-line	40	165–216	7–11	Muscle

*Age class was assessed based on the findings of Cort (1).

[†]Annual samples were pooled for the genetic data analysis based on the absence of significant interannual genetic differentiation (estimated as pairwise F_{ST} s). Details are reported in [SI Text](#).

[‡]The age class of this historical sample was assessed by vertebral ring counting in a subset of 10 individuals. Age marks were read on caudal peduncle vertebrae, which were sawed along a sagittal plane. Sections were stained using silver nitrate staining (2).

[§]The fork length (FL) was calculated using the equation $HCF = 0.299FL - 2.123$ given by Cort (3), where HCF is the height of the caudal fin. Individual HCF was measured from historical caudal fin specimens ([Fig. S1B](#)).

1. Cort JL (1991) Age and growth of the bluefin tuna *Thunnus thynnus* (L.) of the Northeast Atlantic. *Collective Volume of Scientific Papers* 35:213–230.
2. Stevens JD (1975) Vertebral rings as a means of age determination in the blue shark (*Prionace glauca* L.). *J Mar Biol Assoc UK* 55:657–665.
3. Cort JL (1990) Biology and fishery of the Atlantic Bluefin tuna, *Thunnus thynnus*, in the Cantabric Sea (Translated from Spanish). *Publicaciones Especiales IEO 4 (Malaga, Spain)*.

Table S2. Variation of summary statistics at the eight microsatellite loci across *T. thynnus* samples

	Sample				Locus			
	Ttho4	Tth5	Tth10	Tth34	Tth1-31	Tth208	Tth157	Tth62
HADR								
<i>n</i>	69	69	68	69	68	68	67	68
<i>a</i>	11	3	2	14	16	22	6	13
aR	9.13	2.92	2	10.90	12.09	16.39	5.43	11.15
aS	124-170	123-131	116-120	99-155	90-128	140-196	117-127	81-115
H _E	0.83	0.41	0.46	0.85	0.88	0.88	0.70	0.83
H _O	0.68	0.46	0.47	0.71	0.72	0.68	0.60	0.78
HW	0.00*	0.63	1.00	0.05*	0.00*	0.00	0.00	0.24
HSTY								
<i>n</i>	30	30	30	30	29	27	30	30
<i>a</i>	10	3	2	10	9	19	8	15
aR	9.60	2.90	2	9.88	8.92	19	7.78	14.66
aS	136-170	123-131	116-120	95-151	92-126	140-202	105-125	83-117
H _E	0.81	0.41	0.44	0.85	0.85	0.93	0.68	0.88
H _O	0.50	0.40	0.43	0.83	0.79	0.63	0.67	0.67
HW	0.00*	1.00	1.00	0.01	0.00	0.00	0.49	0.00
HCWM								
<i>n</i>	107	105	107	107	107	107	107	107
<i>a</i>	8	3	2	11	16	24	5	7
aR	5.15	2.99	2	7.86	11.65	16.66	4.93	5.80
aS	134-148	123-131	116-120	103-147	90-130	140-220	117-125	91-103
H _E	0.65	0.57	0.41	0.62	0.86	0.90	0.73	0.70
H _O	0.36	0.87	0.56	0.62	0.76	0.81	0.96	0.81
HW	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CADR								
<i>n</i>	73	71	73	73	73	71	73	71
<i>a</i>	16	3	5	17	18	26	7	13
aR	12.90	2.91	3.64	12.99	14.04	18.25	5.34	10.17
aS	130-170	123-131	112-136	103-183	90-128	140-206	117-129	83-113
H _E	0.82	0.47	0.51	0.83	0.89	0.92	0.66	0.84
H _O	0.75	0.42	0.55	0.73	0.84	0.70	0.51	0.79
HW	0.05	0.13	0.00	0.02	0.12	0.00	0.00	0.51
CSTY								
<i>n</i>	39	39	39	39	39	38	38	39
<i>a</i>	12	3	2	12	17	19	6	12
aR	10.86	2.69	2	11.38	15.03	17.30	5.13	11.02
aS	130-170	123-131	116-120	107-187	92-140	140-206	117-127	83-115
H _E	0.85	0.41	0.43	0.82	0.92	0.91	0.48	0.84
H _O	0.77	0.46	0.56	0.92	0.92	0.87	0.58	0.85
HW	0.24	0.17	0.07	0.00	0.15	0.00	0.05	0.15
CLIG								
<i>n</i>	35	35	36	36	36	36	36	36
<i>a</i>	11	3	2	14	13	18	6	13
aR	10.03	3	2	12.48	12.19	16.34	5.75	11.69
aS	136-168	123-131	116-120	103-163	92-130	140-206	119-129	85-123
H _E	0.81	0.54	0.48	0.84	0.90	0.92	0.70	0.87
H _O	0.83	0.43	0.44	0.86	0.86	0.69	0.61	0.78
HW	0.64	0.11	0.73	0.51	0.39	0.01	0.03	0.02
CSAR								
<i>n</i>	29	28	29	29	29	29	29	28
<i>a</i>	12	3	2	11	10	17	4	10
aR	11.58	3	2	10.85	9.86	16.64	4	9.89
aS	124-168	123-131	116-120	103-147	92-126	140-208	119-127	83-109
H _E	0.82	0.49	0.41	0.81	0.83	0.92	0.45	0.85
H _O	0.90	0.32	0.34	0.79	0.72	0.76	0.41	0.82
HW	0.44	0.09	0.64	0.22	0.77	0.00	0.29	0.04
CALG								
<i>n</i>	39	39	39	38	38	39	39	39
<i>a</i>	13	3	2	17	15	18	6	11
aR	11.90	3	2	14.07	12.87	15.96	5.79	10.26

Table S2. Cont.

	Sample					Locus		
	Ttho4	Tth5	Tth10	Tth34	Tth1-31	Tth208	Tth157	Tth62
aS	136-168	123-131	116-120	103-187	90-132	140-216	119-129	83-115
H _E	0.87	0.53	0.49	0.75	0.87	0.91	0.62	0.82
H _O	0.79	0.38	0.54	0.74	0.84	0.69	0.62	0.90
HW	0.11	0.07	0.74	0.78	0.07	0.00	0.87	0.73
CALB								
n	40	40	40	40	40	38	40	40
a	11	3	2	13	13	18	4	10
aR	10.03	2.99	2	11.87	11.81	16.55	4	8.81
aS	138-170	123-131	116-120	103-155	92-138	140-194	119-125	83-111
H _E	0.80	0.49	0.51	0.83	0.90	0.93	0.50	0.74
H _O	0.70	0.60	0.58	0.80	0.80	0.87	0.40	0.65
HW	0.08	0.01	0.52	0.98	0.00	0.18	0.01	0.00

a, alleles; aR, allelic richness per locus and sample; aS, allele size range; H_E, expected heterozygosity; H_O, observed heterozygosity; HW, Hardy-Weinberg equilibrium test.

*Single-locus Hardy-Weinberg equilibrium test (HW) that reaches the equilibrium after applying Microchecker's correction for null alleles to all samples except HCWM.

Table S3. Long-term estimates of θ , N_e , and growth rate in the *T. thynnus* samples

Sample	θ		N_e	Growth rate	
	Estimate	95% CI		Estimate	95% CI
HADR	29.7	21.7, 40.4	74,170	-0.106	-0.134, 0.113
HSTY	39.9	18.3, 44.0	99,670	-0.011	-0.026, 0.197
CADR	35.7	27.0, 47.0	89,250	-0.094	-0.100, 0.130
CSTY	28.0	24.0, 45.4	70,000	-0.147	-0.152, 0.047
CLIG	31.3	22.0, 45.0	78,300	-0.025	-0.043, 0.147
CSAR	22.3	16.2, 39.7	55,750	-0.120	-0.143, 0.076
CALG	29.3	20.6, 42.0	73,120	-0.001	-0.021, 0.217
CALB	21.9	16.7, 34.4	54,750	-0.017	-0.041, 0.198

N_e is obtained using an intermediate value of mutation rate for microsatellites ($\mu = 10^{-4}$).

